



# Blocking TrkB's Effectors Reveal Benefits of the Road Not Taken

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## TrkB-Shc Signaling Protects Against Hippocampal Injury Following Status Epilepticus

Huang YZ, He XP, Krishnamurthy K, McNamara JO. *J Neurosci.* 2019;39(23):4624-4630. doi:10.1523/JNEUROSCI.2939-18.2019. Epub March 29, 2019. PMID: 30926745

Temporal lobe epilepsy (TLE) is a common and commonly devastating form of human epilepsy for which only symptomatic therapy is available. One cause of TLE is an episode of de novo prolonged seizures (status epilepticus [SE]). Understanding the molecular signaling mechanisms by which SE transforms a brain from normal to epileptic may reveal novel targets for preventive and disease-modifying therapies. Status epilepticus-induced activation of the brain-derived neurotrophic factor receptor tyrosine kinase B (TrkB) is one signaling pathway by which SE induces TLE. Although activation of TrkB signaling promotes development of epilepsy in this context, it also reduces SE-induced neuronal death. This led us to hypothesize that distinct signaling pathways downstream of TrkB mediate the desirable (neuroprotective) and undesirable (epileptogenesis) consequences. We subsequently demonstrated that TrkB-mediated activation of phospholipase C $\gamma$ 1 is required for epileptogenesis. Here, we tested the hypothesis that the TrkB-Shc-Akt signaling pathway mediates the neuroprotective consequences of TrkB activation following SE. We studied measures of molecular signaling and cell death in a model of SE in mice of both sexes, including wild-type and TrkBShc/Shc mutant mice in which a point mutation (Y515F) of TrkB prevents the binding of Shc to activated TrkB kinase. Genetic disruption of TrkB-Shc signaling had no effect on severity of SE yet partially inhibited activation of the prosurvival adaptor protein Akt. Importantly, genetic disruption of TrkB-Shc signaling exacerbated hippocampal neuronal death induced by SE. We conclude that therapies targeting TrkB signaling for preventing epilepsy should spare TrkB-Shc-Akt signaling and thereby preserve the neuroprotective benefits. Significance Statement: Temporal lobe epilepsy is a common and devastating form of human epilepsy that lacks preventive therapies. Understanding the molecular signaling mechanisms underlying the development of TLE may identify novel therapeutic targets. Brain-derived neurotrophic factor signaling through TrkB receptor tyrosine kinase is one molecular mechanism promoting TLE. We previously discovered that TrkB-mediated activation of phospholipase C $\gamma$ 1 promotes epileptogenesis. Here, we reveal that TrkB-mediated activation of Akt protects against hippocampal neuronal death in vivo following status epilepticus. These findings strengthen the evidence that desirable and undesirable consequences of SE-induced TrkB activation are mediated by distinct signaling pathways downstream of this receptor. These results provide a strong rationale for a novel therapeutic strategy selectively targeting individual signaling pathways downstream of TrkB for preventing epilepsy.

## Commentary

Despite the introduction of dozens of medications that can control seizures in many patients with epilepsy, the development of a therapy that can prevent epilepsy development or improve disease course remains elusive. Animal studies targeting the brain-derived neurotrophic factor (BDNF) receptor tropomyosin-related tyrosine kinase B (TrkB) have shown promising results in epilepsy models. Brain-derived neurotrophic factor is released following epileptogenic brain injuries, such as status epilepticus, leading to a dramatic increase in TrkB activation.<sup>1</sup> Activated TrkB, in turn, can act through a variety of signaling pathways that promote neuronal remodeling,

increase synaptic strength, and enhance neuronal activity. Furthermore, infusing BDNF directly into the brains of normal rodents can promote seizures,<sup>2</sup> and enhancing TrkB activation can facilitate epileptogenesis.<sup>3</sup> Decreasing BDNF signaling and blocking TrkB activation, on the other hand, has antiepileptogenic effects in the kindling, traumatic brain injury, and status epilepticus models of epilepsy.<sup>4-7</sup>

While rodent studies implicate TrkB activation in the development of epilepsy, the diversity of TrkB functions serves as an impediment to targeting the pathway for clinical translation. TrkB is a transmembrane protein with an intracellular tyrosine kinase domain. Upon binding BDNF, TrkB dimerizes and



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phosphorylates 3 tyrosine residues in the autoregulatory loop of the kinase domain. Once activated, the kinase phosphorylates 2 additional tyrosine residues on the intracellular domain that mediate signaling through phospholipase C- $\gamma$  (PLC- $\gamma$ ), extracellular signal-regulated kinase (ERK), and phosphatidylinositol 3-kinase (PI3K-AKT). Phospholipase C- $\gamma$  initiates inositol tris-phosphate and diacylglycerol signaling, which has a wide range of effects, including promoting synaptic assembly, chemoattraction, and long-term potentiation. Shc-binding, on the other hand, activates the PI3K-AKT and ERK signaling pathways. This also produces a range of effects, including cell survival and axonal growth.<sup>8</sup> Rodent studies targeting TrkB directly block signaling through all pathways and can produce opposing effects from the standpoint of developing epilepsy therapeutics. For example, blocking TrkB beginning shortly after status epilepticus increases acute neuronal loss<sup>9</sup> but mitigates the development of epilepsy and decreases chronic neuronal loss.<sup>7</sup>

To overcome these issues, investigators have worked to develop tools to selectively manipulate the different signaling pathways activated by TrkB. In 2015, Gu and colleagues<sup>9</sup> used a designer peptide to selectively block TrkB-mediated PLC- $\gamma$  signaling following infusion of kainic acid into the amygdala to induce status epilepticus. Peptide treatment beginning immediately after the termination of status epilepticus with diazepam did not affect the severity of status or the amount of status-induced cell loss, but significantly reduced the severity of subsequently developing epilepsy in the animals, evident as a 90% reduction in seizure frequency.

To further explore the role of the different TrkB-activated signaling pathways, Huang and colleagues used the intra-amygdala kainic acid model to induce status epilepticus in TrkB<sup>shc/shc</sup> mice. In these animals, tyrosine 515, which serves as the Shc binding site, was mutated to phenylalanine, thereby preventing phosphorylation and blocking Shc-mediated signaling. Kainic acid-induced status epilepticus in TrkB<sup>shc/shc</sup> mice was of similar duration and severity to wild-type animals, demonstrating that TrkB-Shc signaling does not exert acute antiseizure effects. This finding also serves as an important control for cell death measures, as valid comparisons require that the insults in mutant and wild-type animals be of equal severity. Notably, the lack of an effect of blocking TrkB-Shc signaling on status epilepticus contrasts with studies blocking the PLC- $\gamma$  site before the induction of status epilepticus, which did reduce insult severity.<sup>9</sup> Despite the similar severity of status epilepticus in TrkB<sup>shc/shc</sup> mice, the mutant animals exhibited significantly greater loss of hippocampal CA3 pyramidal cells than wild-type animals. Increased cell loss was associated with a reduction in the seizure-induced increase in phosphorylated AKT. The findings, therefore, are consistent with the known role of PI3K-AKT pathway in promoting cell survival and support the conclusion that activation of TrkB-Shc binding plays a neuroprotective role in status epilepticus.


CA3 pyramidal cells are glutamatergic excitatory neurons that constitute a key component of the hippocampal trisynaptic circuit, in which entorhinal cortex neurons innervate

hippocampal granule cells, granule cells innervate CA3 pyramidal cells, and CA3 pyramidal cells innervate CA1 pyramidal cells. This circuit is critical for episodic and spatial memory and can exhibit extensive loss of component neurons in patients with temporal lobe epilepsy and hippocampal sclerosis. Memory and cognitive deficits experienced by patients with epilepsy are commonly attributed to disruption of this circuit. Preventing neuronal loss in this region, therefore, could reduce epilepsy comorbidities. Yet to be explored is whether blocking TrkB-Shc signaling exacerbates the development or severity of epilepsy. Hippocampal neuron loss is hypothesized to be an instigating factor in epileptogenesis, so treatments that increase neuronal loss—like blocking TrkB-Shc signaling—would be predicted to make epilepsy worse. Curiously, kindling epileptogenesis was not enhanced in TrkB<sup>shc/shc</sup> mice<sup>10</sup>; however, kindling models only the early stages of the epileptogenic process, in which cell loss is minimal. Spontaneous seizure monitoring studies in status epilepticus-exposed TrkB<sup>shc/shc</sup> mice with extensive cell loss would shed light on whether the animals develop a more severe epilepsy. Conversely, it would also be important to assess whether enhancing TrkB-Shc signaling could mitigate neuron loss from status epilepticus and reduce epilepsy severity.

Taken in toto, the literature indicates that activation of the TrkB receptor following status epilepticus can have both beneficial and harmful effects. Naturally, a therapy which could preserve the beneficial effects and block the harmful effects would be ideal. The exciting conclusion of the Huang et al study is that these different effects are mediated by distinct signaling pathways, which can be experimentally dissociated. This is an encouraging result for the development of novel therapeutics to prevent and treat epilepsy. The findings also help to elucidate one aspect of the complexity surrounding BDNF-TrkB signaling in epilepsy. Translation of these research findings to clinical practice, however, is likely to require further investigation into a number of additional complexities. Of particular note, TrkB signaling is important in both excitatory and inhibitory neurons. While synaptic strengthening in excitatory neurons would enhance network hyperactivation, the same process in inhibitory neurons might lead to an overall reduction in brain excitability. Indeed, treating animals with a partial TrkB receptor agonist in the cortical undercut model of epilepsy increased the expression of inhibitory synaptic markers and reduced epileptiform activity. Conversely, blocking TrkB activation prevented inhibitory neuron sprouting.<sup>11</sup> Complexity at the receptor level, therefore, is compounded by complexity at the cellular level. Differences in epilepsy models are also likely to be critical. Epilepsy syndromes that result primarily from increased excitability, for example, might benefit more from the use TrkB antagonists, while TrkB agonists might be effective in promoting neuronal growth or survival in epilepsies driven by interneuron dysfunction or loss. The development of agents to selectively target the different signaling pathways downstream of TrkB could be an opportunity area here, as desired effects (eg, synaptic

strengthening vs neuronal survival) could be matched to disease etiology.


The road to epilepsy has always followed many routes simultaneously. New tools now allow us to choose between routes and that could make all the difference.

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### References

1. Binder DK. Neurotrophins in the dentate gyrus. *Prog Brain Res.* 2007;163:371-397.
2. Scharfman HE, Goodman JH, Sollas AL, Croll SD. Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. *Exp Neurol.* 2002;174(2):201-214.
3. Heinrich C, Lähäinen S, Suzuki F, et al. Increase in BDNF-mediated TrkB signaling promotes epileptogenesis in a mouse model of mesial temporal lobe epilepsy. *Neurobiol Dis.* 2012;42(1):35-47.
4. Lähäinen S, Pitkänen A, Saarelainen T, Nissinen J, Koponen E, Castrén E. Decreased BDNF signaling in transgenic mice reduces epileptogenesis. *Eur J Neurosci.* 2002;15(4):721-734.
5. He XP, Kotloski R, Nef S, Luikart BW, Parada LF, McNamara JO. Conditional deletion of TrkB but not BDNF prevents epileptogenesis in the kindling model. *Neuron.* 2004;43(1):31-42.
6. Aungst S, England PM, Thompson SM. Critical role of trkB receptors in reactive axonal sprouting and hyperexcitability after axonal injury. *J Neurophysiol.* 2013;109(3):813-824.
7. Liu G, Gu B, He XP, et al. Transient inhibition of TrkB kinase after status epilepticus prevents development of temporal lobe epilepsy. *Neuron.* 2013;79(1):31-38.
8. Deinhardt K, Chao MV. Trk receptors. *Handb Exp Pharmacol.* 2014;220:103-119.
9. Gu B, Huang YZ, He XP, Joshi RB, Jang W, McNamara JO. A peptide uncoupling BDNF receptor TrkB from phospholipase C $\gamma$ 1 prevents epilepsy induced by status epilepticus. *Neuron.* 2015;88(3):484-491.
10. He XP, Minichiello L, Klein R, McNamara JO. Immunohistochemical evidence of seizure-induced activation of trkB receptors in the mossy fiber pathway of adult mouse hippocampus. *J Neurosci.* 2002;22(17):7502-7508.
11. Gu F, Parada I, Yang T, Longo FM, Prince DA. Partial TrkB receptor activation suppresses cortical epileptogenesis through actions on parvalbumin interneurons. *Neurobiol Dis.* 2018;113:45-58.